TITLE: CRISPR1-RFLP AS AN ALTERNATIVE METHOD FOR MOLECULAR TYPING OF SEROTYPE III GROUP B STREPTOCOCCUS FROM BOVINE AND HUMAN ORIGINS

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ABSTRACT

Group B *Streptococcus* (GBS) of serotype III is considered an important cause of invasive disease in human neonates and contagious mastitis in bovines. DNA-based typing methods, especially MLST, are useful to track and unravel the molecular epidemiology of potentially pathogenic strains circulating worldwide. However, MLST relies on gene sequencing and is not widely accessible in low-and-middle income countries, like Brazil. Thus, cost-effective alternative methods have been proposed for GBS strain-typing, including CRISPR1-RFLP. We assessed the potential of CRISPR1-RFLP as a molecular typing method of serotype III GBS strains from human and bovine origins. Fifteen isolates recovered from bovine milk and seventeen isolates from adult (n=4) and neonatal (n=3) invasive disease specimens or anovaginal secretion (n=10) were evaluated. They were obtained in the South and Southeast regions of Brazil from 1987 to 2020. CRISPR1 elements were identified by PCR, subjected to RFLP (*Dde*I enzyme), and fingerprinting profiles were analyzed with BioNumerics v7.3.6. CRISPR1-RFLP distributed the 32 isolates in 4 clusters (A-D) and 1 singleton (Simpson's Index of Diversity=0.841). Clusters A and C included GBS isolates from cattle and anovaginal carriage, mostly belonging to ST19 and ST23. Both ST19 and ST23 are considered versatile lineages frequently encountered in both human and bovine populations. Cluster B included all GBS isolates from invasive disease, which belonged to ST17; while cluster D was composed exclusively by bovine isolates and associated with ST91. Serotype III/ST17 GBS is a hypervirulent clone exclusively found in humans and recognized as the main cause of GBS neonatal meningitis. In contrast, ST91 is a double locus variant of ST61, and part of a the well-adapted and exclusively bovine CC61/67. All 4 clusters included GBS isolates recovered in the 1980/90's and 2000/10's, indicating that these strains have been circulating in Brazil for at least 2 decades. Serotype III GBS is widespread worldwide among animal and human hosts both in colonization and disease, highlighting its interspecies transmission potential, and contagiousness. Pathogen surveillance can aid in the detection of transmission routes, reservoirs, and distribution of strains across the globe, and CRISPR1-RFLP may be a cost-effective and accessible technique for detecting and differentiating serotype III clones adapted to humans, bovines or both hosts in Brazil.

Keywords: Group B *Streptococcus*, molecular epidemiology, typing method, strain-typing, CRISPR1-RFLP.